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Consejo Superior De Investigaciones Cientificas
Calle Serrano 117
E-28006 Madrid
Spain

8649089001

Patents ADP number *(if you know it)* — (see attached)

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4. Title of the invention

MAGNETIC NANOPARTICLES

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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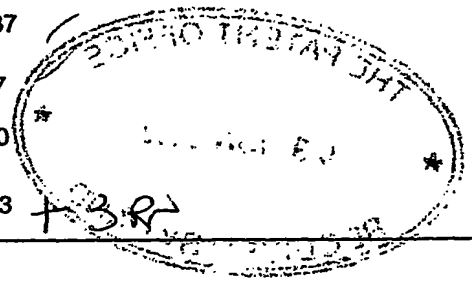

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Magnetic Nanoparticles

Field of the Invention

The present invention relates to magnetic nanoparticles, and in particular to magnetic nanoparticles having immobilised ligands and their use in studying the interaction of these ligands with other species. The present invention further relates to applications of the nanoparticles, for example for screening, diagnosis and therapy.

Background of the Invention

The development of methodologies to produce nanoparticles with bio-responsive properties has opened the way for producing useful tools for molecular diagnostics, therapeutics and biotechnology [1]. Metal, semiconductor and magnetic colloidal nanoparticles are presently under intensive study for potential applications [2].

Nanoparticles containing paramagnetic materials such as iron oxide have been made which exhibit unusually strong magnetic properties under external magnetic fields. These magnetic nanoparticles can be used in many biomedical applications, including cell separation, *in vivo* cell and tissue labelling, contrast enhancement in magnetic resonance imaging, tumour targeting, hyperthermia therapies and drug delivery.

For such applications, the nanoparticles should preferably be small enough to avoid provoking an immune response and to be taken up by cells, where necessary. It is also useful if the size of the particles can be controlled as the particles should be of approximately the same size so they display the same magnetic

properties. The particles should also preferably be chemically stable, so they are not broken down by the body.

In is also preferred that magnetic nanoparticles for use in biomedicine are soluble, especially in water, in order that they may be stored and administered effectively. Ideally, such particles would be stable in solution and would not aggregate, either when stored before use or in the body. Magnetic nanoparticles tend to clump together in solution because they attract each other. If this happened in the body it could impede blood flow and potentially be dangerous; in colloidal solution it would make the colloid difficult to use.

Previously, commercially available iron oxide particles have been used in cell sorting and separation [3]. Monodisperse magnetic nanoparticles of Fe/Pt [4], Co and Co/Fe [5], Fe [6], and iron oxides [7] have recently been synthesised by solution chemistry for materials applications.[8]. Iron oxide nanoparticles coated with cross-linked dextran to prevent clumping have also been described, see for example WO 03/005029.

Ideally, the magnetic nanoparticles are made of elemental magnetic metal rather than metal oxide, as elemental metal is a better enhancer of magnetic imaging. However, such nanoparticles are often chemically unstable, as the metal may oxidise. One possibility for increasing the chemical stability of magnetic nanoparticles is to synthesise them from a magnetic metal with a passive metal to stabilise the magnetic metal.

US 2002/0068187 discloses surfactant protected gold-iron core-shell nanoparticles synthesised by means of reverse micelles. However, this method is complex, requiring three synthesis steps. The multi-layered composition of the resulting particles also increases the lower size limit for the particles, which can be a disadvantage if very small particles are required [14].

US Patent No:6,254,662 discloses use of FePt and CoPt alloy nanoparticles to form nanocrystalline thin films on a solid surface, for use in making ultra-high density recording media. Other uses of the films are mentioned in the patent, including use as magnetic bias films and magnetic tips for magnetic force microscopy, but biomedical applications are not envisaged.

For many of the applications described above, it is necessary to link the nanoparticles to biologically active molecules such as ligands that bind to intracellular or extracellular molecules. Such ligands may for example be carbohydrate, nucleic acid or protein.

US Patent No: 6,514,481 provides iron oxide nanoparticles coated with a silica shell, which shell is linked to a targeting molecule such as a peptide via a spacer molecule. WO 02/098364 and WO 01/19405 disclose magnetic metal oxide nanoparticles coated with dextran and functionalised with peptides and oligonucleotides. Similar strategies have been used to prepare nanoparticles for intracellular labelling [9] and as nanosensors.[10]. All these methods are time-consuming multi-step methods requiring that the nanoparticles be coated with dextran or silica, the coated nanoparticles

be functionalised so they will bind the ligand, and finally that the ligand be bound to the nanoparticles.

WO 02/32404 discloses water soluble nano-tools for studying carbohydrate mediated interactions [11][12]. These tools are gold glyconanoparticles and cadmium sulphide glyco-nanodots incorporating carbohydrate antigens. These water soluble gold and semiconductor nanodots are stable for months in physiological solutions and present exceptionally small core sizes. They are resistant to glycosidases and do not present cytotoxicity. They are also useful platforms for basic studies of carbohydrate interactions [13] and are tools for biotechnological and biomedical applications. However, these nanoparticles are not magnetic.

There is therefore a continuing need in the art for stable magnetic nanoparticles which are bound to ligands to make them suitable for biomedical uses, which can be synthesised to a desired size, and which can be produced by a simple, reliable synthesis method.

Summary of the Invention

Broadly, the present invention provides materials and methods for producing magnetic nanoparticles suitable for use in biomedical applications. In particular, the present invention provides magnetic nanoparticles which are employed as a substrate for immobilising a plurality of ligands. The ligands may comprise carbohydrate groups, peptides, protein domains, nucleic acid segments or fluorescent groups. These nanoparticles can then be used to study ligand mediated interactions, e.g. with other carbohydrates, proteins or nucleic acids, and as therapeutics and diagnostic reagents. In some

embodiments, the particles have the further advantage that they are soluble, e.g. in water and a range of organic solvents, and can be used in a variety of homogeneous application formats.

The inventors have now developed magnetic nanoparticles with size in the nanometre scale which form stable colloidal aqueous solutions (ferrofluids). The methods described herein constitute a simple and versatile approach by which neoglycoconjugates of significant carbohydrates are covalently linked to gold/iron clusters as a method for tailoring stable, water-soluble, magnetic glyconanoparticles with globular shapes and highly polyvalent carbohydrate surfaces. The methodology also allows the attachment of many other molecules directly to the nanocluster.

Accordingly, in a first aspect, the present invention provides a particle comprising a magnetic core, such as a metallic core, linked to a plurality of ligands. The ligands may comprise carbohydrate groups, peptides, protein domains, nucleic acid segments or other biological macromolecules. The ligands may additionally or alternatively comprise fluorescent groups.

Preferably, the magnetic core comprises passive metal atoms and magnetic metal atoms, and the ratio of passive metal atoms to magnetic metal atoms in the core is between about 5:0.1 and about 2:5. More preferably, the ratio is between about 5:0.1 and about 5:1.

As used herein, the term 'passive metals' refers to metals which do not show magnetic properties and are chemically stable to oxidation.

The passive metals of the invention may be diamagnetic. 'Diamagnetic' refers to materials with all paired electrons which thus have no permanent net magnetic moment per atom. 'Magnetic' materials have some unpaired electrons and are positively susceptible to external magnetic fields - that is, the external magnetic field induces the electrons to line up with the applied field, so the magnetic moments of the electrons are aligned.

Magnetic materials may be paramagnetic, superparamagnetic or ferromagnetic. Paramagnetic materials are not very susceptible to external magnetic fields and do not retain their magnetic properties when the external magnetic field is removed. Ferromagnetic materials are highly susceptible to external magnetic fields and contain magnetic domains even when no external magnetic field is present, because neighbouring atoms cooperate so their electron spins are parallel. External magnetic fields align the magnetic moments of neighbouring domains, magnifying the magnetic affect. Very small particles of materials that normally have ferromagnetic properties are not ferromagnetic, as the cooperative effect does not occur in particles of 300nm or less so the material has no permanent magnetism. However, the particles are still very susceptible to external magnetic fields and have strong paramagnetic properties, and are known as superparamagnetic. Preferably, the nanoparticles of the invention are superparamagnetic.

In one embodiment, the nanoparticle consists of a core comprising passive metal atoms and magnetic metal atoms, which core is covalently linked to a plurality of ligands. Preferably, the ratio of passive metal atoms to

magnetic metal atoms in the core is between about 5:0.1 and about 2:5. More preferably, the ratio is between about 5:0.1 and about 5:1.

In a further aspect, the present invention provides compositions comprising populations of one or more of the above defined particles. In some embodiments, the populations of nanoparticles may have different densities of the same or different ligands attached to the core.

In a further aspect, the present invention provides the above defined particles for use in a method of medical treatment.

In a further aspect, the present invention provides the use of the above defined particles for the preparation of a medicament for the treatment of a condition ameliorated by the administration of the ligand. By way of example, this may occur as the ligand blocks a carbohydrate mediated interaction that would otherwise tend to lead to a pathology.

In this embodiment, the present invention has advantages over prior art approaches for treating conditions involving carbohydrate mediated interactions. As described above, typically the interactions are polyvalent whereas the agent used to treat the interactions are often only capable of modulating one or a few of these interactions. This has the result that it is difficult to deliver an agent to the site of the interaction which is capable of reliably modulating the interaction for the desired therapeutic effect. In contrast to this problem, the present invention provides agents having a plurality of ligands for modulating the

carbohydrate mediated interactions, potentially overcoming the difficulty in modulating the polyvalent interactions.

In preferred embodiments, the mean diameter of the core, preferably the metallic core, is between 0.5 and 100nm, more preferably between 1 and 50nm, more preferably between 1 and 20nm. Still more preferably, the mean diameter of the core is below 2nm. The mean diameter can be measured using techniques well known in the art such as transmission electron microscopy.

The core material can be a metal and may be formed of more than one type of atom. Preferably, the core material is a composite or an alloy of a passive metal and a magnetic metal. Preferred passive metals are Au, Ag, Pt or Cu and preferred magnetic metals are Fe and Co, with the most preferred composite being Au/Fe. Other composites or alloys may also be used.

Previously described magnetic nanoparticles for biological applications are almost always made from a magnetic metal oxide, usually iron oxide (magnetite). Nanoparticles comprising Fe and Au have been made, as described above, but have not been used for biological applications or bound to biologically active molecules. These nanoparticles are synthesised as a "nano-onion" comprising a gold core surrounded by an iron shell which is coated with gold to prevent oxidation. The nanoparticles described herein, which have a heterogeneous core comprising both gold and iron atoms, are an improvement over the previously described particles because they can be synthesised in a single

simple step, rather than requiring multiple synthesis steps to form the different shells of the nano-onion.

The nanoparticles and the results of their interactions can be detected using a number of techniques well known in the art. These can range from detecting the aggregation that results when the nanoparticles bind to another species, e.g. by simple visual inspection or by using light scattering (transmittance of a solution containing the nanoparticles), to using sophisticated techniques such as transmission electron microscopy (TEM) or atomic force microscopy (AFM) to visualise the nanoparticles. A further method of detecting metal particles is to employ plasmon resonance, that is the excitation of electrons at the surface of a metal, usually caused by optical radiation. The phenomenon of surface plasmon resonance (SPR) exists at the interface of a metal (such as Ag or Au) and a dielectric material such as air or water. As changes in SPR occur as analytes bind to the ligand immobilised on the surface of a nanoparticle changing the refractive index of the interface. A further advantage of SPR is that it can be used to monitor real time interactions. As mentioned above, if the nanoparticles includes or is doped with atoms which are NMR active then this technique can be used to detect the particles, both in vitro or in vivo, using techniques well known in the art. Nanoparticles can also be detected as described in [18], using a system based on quantitative signal amplification using the nanoparticle-promoted reduction of silver (I) and using a flatbed scanner as a reader. Fluorescence spectroscopy can be used if the nanoparticles include ligands combining carbohydrate groups and fluorescent probes.

Also, isotopic labelling of the carbohydrate can be used to facilitate their detection.

The ligand linked to the core may comprise one or more carbohydrate (saccharide) groups, e.g. comprising a polysaccharide, an oligosaccharide or a single saccharide group. The ligand may be also be a glycanoconjugate such as a glycolipid or a glycoprotein. In addition to the carbohydrate group, the ligand may additionally comprise one or more of a peptide group, a protein domain, a nucleic acid molecule (e.g. a DNA segment) and/or a fluorescent probe.

In another embodiment, the ligand may be a peptide or a protein. These may be peptides which binds to receptors on a cell, or they may be antibodies, or therapeutic proteins.

In a further embodiment, the ligand may be a nucleic acid molecule. The nucleic acid may be an oligonucleotide probe that binds to a sequence within the cell. Alternatively, the nucleic acid may comprise an encoding gene sequence for delivery to a cell.

The particles may have more than one species of ligand immobilised thereon, e.g. 2, 3, 4, 5, 10, 20 or 100 different ligands. Alternatively or additionally a plurality of different types of particles can be employed together.

In preferred embodiments, the mean number of ligands linked to an individual metallic core of the particle is at least 20 ligands, more preferably at least 50 ligands, and most preferably 60 ligands.

Preferably, the ligands are attached covalently to the core of the particles. Protocols for carrying this out are known in the art, although the work described herein is the first report of the reactions being used to covalently bond ligands to the core of the particle. This may be carried out by reacting ligands with reductive end groups with gold and iron under reducing conditions. A preferred method of producing the particles employs thiol derivatised carbohydrate moieties to couple the ligands to particles. Thus, in one aspect, the present invention provides a method of preparing the above defined particles, the method comprising:

synthesizing a sulphide derivative of the ligand;

reacting the sulphide derivatised ligand with a ferric salt and $HAuCl_4$ (tetrachloroauric acid) in the presence of reducing agent to produce the particles. A preferred iron salt is $FeCl_3$.

In a preferred embodiment, the ligand is derivatised as a protected disulphide. Conveniently, the disulphide protected ligand in methanol or water can be added to an aqueous solution of tetrachloroauric acid. A preferred reducing agent is sodium borohydride. Other preferred features of the method are described in the examples below.

The present invention provides a way of presenting a spherical array of ligands having advantages over other types of array proposed in the prior art. In particular, the nanoparticles are soluble in most organic solvents and especially water. This can be used in their purification and importantly means that they can be used in solution for presenting the ligand immobilised on the surface of the particle. The fact that the nanoparticles

are soluble has the advantage of presenting the ligands in a natural conformation. For therapeutic applications, the nanoparticles are non-toxic, soluble and stable under physiological conditions.

Magnetic nanoparticles in solution form magnetic colloids known as ferrofluids. Ferrofluids have the fluid properties of a liquid and the magnetic properties of a solid. They have a range of applications, as described below. The main problem encountered with ferrofluids known in the art is their lack of stability: because the magnetic particles attract each other, they will agglomerate after a certain time. Previously used methods of preventing agglomeration include coating the particles with surfactants, crosslinking polymers or polysaccharides. If the nanoparticle is to be bound to a ligand or targeting molecule, a further synthesis step is required.

The particles of the present invention are highly soluble in water and are thus ideal for making ferrofluids. Moreover, the resulting ferrofluids are extremely stable and can be kept for many months without aggregating. Ferrofluids of the invention have been kept for a year with no sign of aggregation. The methods of the present invention allow magnetic nanoparticles that are stable and already bound to functional ligands to be synthesised in a single reaction, rather than requiring the particles first to be coated and then bound to ligands.

Stability may be assessed by eye - a colloidal solution remains transparent in the absence of agglomeration, but becomes opaque once it starts to agglomerate. Alternatively, the presence of flocculation may be

determined by transmission electron micrography (TEM), or by comparing the proton NMR spectra of the particles in deuteron water with those of freshly prepared nanoparticles. Preferably, the magnetic particles will show no sign of agglomeration for at least a year after preparation.

In the method described herein, the formation of the Au/Fe cluster and the covalent linking of the ligand is a simultaneous process, so that the presence of the neoglycoconjugate controls the shape and size of the nanoclusters. The Au/Fe glyconanoparticles prepared in this way have a core of less than 2 nm diameter, which is smaller than any of the magnetic nanoparticles known in the art. Superparamagnetic behaviour is shown at all temperatures and superconducting quantum interference device (SQUID) measurements indicate also the existence of a ferromagnetic component at room temperature. This anomalous magnetic property may be of importance for imaging and cell separations.

The following examples of application for the magnetic nanoparticles and ferrofluids are provided by way of illustration and not limitation to support the wide applicability of the technologies described herein.

In one aspect of the invention, the magnetic properties of the nanoparticles of the invention are exploited in cell separation techniques which eliminate the need for columns or centrifugation. This permits a highly pure population of cells to be obtained quickly and easily. In one embodiment, the nanoparticles may be linked to ligands which specifically bind a receptor on the cell of interest. The nanoparticles may then be added to a cell

suspension and the particle-bound cells separated from the rest of the suspension by application of a magnetic field.

This is a highly sensitive as well as efficient method which can be used in many applications, for example in diagnosis of tumours by testing body fluids for the presence of tumour cells. The sensitivity of the technique is a great advantage in this respect.

In a further aspect, the present invention provides a method of determining whether an interaction with a ligand occurs, the method comprising contacting one or more species of ligand-bound nanoparticles with a candidate binding partner and determining whether binding takes place.

In a further aspect, the present invention provides a method of screening for substances capable of binding to a ligand, the method comprising:

contacting particles having a core comprising a passive metal and a magnetic metal, which core is covalently linked to a plurality of the ligands, with one or more candidate compounds; and

detecting whether the candidate compounds binds to the ligand. Preferably, the ratio of passive metal atoms to magnetic metal atoms in the core is between about 5:0.1 and about 2:5. More preferably, the ratio is between about 5:0.1 and about 5:1.

In a further aspect, the present invention provides a method of determining the presence in a sample of a substance capable of binding to a ligand, the method comprising contacting the sample with nanoparticles

linked to the ligand and determining whether binding takes place. The method may be used to determine the presence or amount of one or more analytes in a sample, e.g. for use in assisting the diagnosis of a disease state associated with the presence of the analyte. The presence of analytes may be signalled by the formation of analyte-nanoparticle aggregates, the presence of which can be detected by measuring the relaxation properties of the fluid in the sample. A change in the relaxation properties indicates the presence of aggregates and hence target molecules.

Where the ligand is a carbohydrate, a range of different carbohydrate mediated interactions are known in the art and could be studied or modulated using the nanoparticles disclosed herein. These include leukocyte-endothelial cell adhesion, carbohydrate-antibody interactions, carbohydrate-protein bacterial and viral infection, immunological recognition of tumour cells, tumour cells-endothelial cells (e.g. to study metastasis) and foreign tissue and cell recognition.

In another aspect, the magnetic nanoparticles and ferrofluids of the invention can be used to treat cancer. Magnetic nanoparticles may be used for hyperthermic treatment of tumours, in which magnetic nanoparticles are injected into tumours and subjected to a high frequency AC or DC magnetic field. Alternatively, near IR light may be used. The heat thus generated by the relaxation magnetic energy of the magnetic material kills the tumour tissue around the particles. In one embodiment of the present invention, tumour cells may be specifically targeted by incorporating tumour-specific antigens into the nanoparticles. This allows tumours not easily

reached by injection to be targeted by the therapeutic particles, and avoids killing of normal healthy cells.

For a given excitation frequency, there exists an optimum nanoparticle size that yields a maximum specific absorption rate (SAR) and thus most efficient heating. This technique thus requires magnetic nanoparticles with a narrow core size distribution, to maximise the efficiency of the therapy and minimise the amount of ferrofluid to be administered. The magnetic nanoparticles of the invention are thus particularly well suited to this application, as the synthesis method enables the size of the nanoparticles to be closely controlled.

In another embodiment, the nanoparticles may be linked to therapeutically active substances such as antibodies or tumour-killing drugs. The magnetic properties of the nanoparticles can also be used to target tumours, by using a magnetic field to guide the nanoparticles to the tumour cells. However, use of magnetic field alone to direct nanoparticles to tumour cells is not always feasible or accurate, so the present invention provides an advantage by enabling the nanoparticles to be specifically directed to tumour cells via tumour-specific ligands. This will allow less drug to be used and reduce the chance of side effects, as the drug is directed only to the cells where it is needed and not to healthy cells.

In a further aspect, the magnetic nanoparticles of the invention may be used to improve the quality of magnetic resonance imaging (MRI). MRI does not always provide enough contrast to enable structures such as tumours to be efficiently viewed, but the images obtained can be enhanced by using magnetic nanoparticles as contrast

media. The enhanced sensitivity thus obtained enables tumours to be detected while still very small. This permits detection of tumours at a very early stage when there is more chance of successful treatment.

Detection of tumour cells in this way can also be combined with hyperthermia: once the tumour cells are identified, laser or near IR light may de directed to the tumour site to kill the cells.

The ligand-bound particles of the present invention can be delivered specifically to tumour cells so even tumour cells which have moved away from the original tumour site may be targeted for therapy.

Embodiments of the present invention which have a core comprising elemental magnetic metal are particularly well suited to imaging applications, as elemental metal is a more efficient enhancer of imaging then metal oxide. The presence of a passive metal in the core is advantageous as it inhibits oxidation of the magnetic metal. The passive metal also increases the biocompatibility of the nanoparticles and permits the core to be bound to ligands, which in addition to their biological uses further protect the magnetic metal from oxidation and reduce the likelihood of agglomeration.

Another advantage of the nanoparticles of the present invention is their exceptionally small size, which makes them more likely to be taken up by cells even when linked to targeting or therapeutic molecules.

In a further aspect, the magnetic nanoparticles of the invention may be used to replace radioactive materials used as tracers for drug delivery. Use of magnetic particles instead of radioactive materials permits drug

delivery to be assessed by measuring magnetic variations, eliminating potential harm from radiation.

In general, it has been a difficult problem in the art to detect or modulate carbohydrate-mediated interactions since the binding of carbohydrates to other species such as proteins or other carbohydrates is very weak and tends to be polyvalent. Thus, for detection the binding is weak and for modulating interaction, monovalent agents have only had a limited success in disrupting polyvalent carbohydrate based interactions.

In embodiments of the invention relating to carbohydrate-carbohydrate interactions, two types of interaction can be identified. In homophilic interactions, identical carbohydrates interact with one another and could be detected by steadily increasing the concentration of particles having a single species of ligands immobilised on their surface until aggregation occurs. This may be detected by light scattering or electronic effects. Heterophilic interactions can be detected by mixing together two or more different nanoparticles and determining the aggregation state of the particles.

Thus, the present invention provides a versatile platform for studying and modulating carbohydrate-mediated interactions. For example, the particles could be used to detect anti-carbohydrate antibodies, detecting the binding of antibody to the ligands on the particle via light scattering to pick up aggregation of the particles, or electric field effects, such as surface plasmon resonance, which would be modified when the metal atoms in the particles cluster together.

The invention thus provides a method of determining whether a carbohydrate mediated interaction occurs, the method comprising contacting one or more species suspected to interact via a carbohydrate mediated interaction with the nanoparticles of the invention , and determining whether the nanoparticles modulate the carbohydrate mediated interaction.

The invention also provides a method of disrupting an interaction between a carbohydrate and a binding partner, the method comprising contacting the carbohydrate and the binding partner with the nanoparticles of the invention, wherein the nanoparticles comprise a carbohydrate group capable of disrupting the interaction of the carbohydrate and the binding partner.

In a further aspect, nanoparticles in which the ligand is an antigen can be administered as a vaccine, e.g. ballistically, using a delivery gun to accelerate their transdermal passage through the outer layer of the epidermis. The nanoparticles can then be taken up, e.g. by dendritic cells, which mature as they migrate through the lymphatic system, resulting in modulation of the immune response and vaccination against the antigen.

Nanoparticles in which the ligand is nucleic acid encoding an antigen may also be administered as a vaccine. Nanoparticles are particularly well suited to such applications because nucleic acid vaccines must enter individual cells to be effective, so it is important that particles small enough to penetrate the cell membrane without damaging the cells be used.

Vaccine delivery guns known in the art power delivery by use of compressed air or gas, usually helium gas. This can be painful and causes weals on the skin. The magnetic nanoparticles of the invention could be used in an alternative delivery system whereby the power for delivering the particles is provided by application of a magnetic field. Reversal of the magnetic field would result in rapid acceleration of the nanoparticles, sufficient to propel them through the outer epidermal layer. This would reduce pain and weal formation resulting from the use of compressed gas.

In a further application, it is known that cell surface carbohydrates act as ligands for viral or bacterial receptors (called adhesins) and that binding of the carbohydrates to the receptors is an event required during infection. Synthetic carbohydrates, e.g. glycoconjugates, that are capable of modulating these interactions can be immobilised in the nanoparticles of the invention and used as reagents to study these interactions and as therapeutics to prevent viral or bacterial infection.

In a further application, the present invention may be useful in the modulation of immune response, e.g. following transplantation. As the immunological recognition of tissue begins with carbohydrate mediated interactions between surface carbohydrates present on transplanted tissue and the components of the host's immune system such as antibodies, so this can be targeted to ameliorate immune reactions that result from this interaction. By way of example the carbohydrate Galα1-3Galβ1-4GlnAc (the 'αGal' epitope) has been implicated as an important antigenic epitope involved in the rejection

of transplanted tissue. Thus, modulation of the interaction of the αGal epitope and the immune system may be a therapeutic target for the nanoparticles described herein.

An alternative approach may be useful in the treatment of cancer as many tumour associated antigens or tumour autocrine factors are carbohydrate based. In this event, the nanoparticles could be provided as vaccines to prime the immune system to produce antibodies which are capable of attacking tumour cells presenting the carbohydrates on their surface. In this regard, it is known that many tumour cells possess aberrant glycosylation patterns which may enable the immune response stimulated by nanoparticles to be directed specifically to tumour cells as opposed to normal, healthy cells. The nanoparticles can also be used to inhibit metastatis in cancer, e.g. through the migration of tumour cells through the endothelial cells.

In a further aspect, the nanoparticles can be used as carriers to raise antibodies capable of specifically binding the ligand. This is particularly advantageous where the ligand is a carbohydrate, as it can be a challenging problem in the art to raise antibodies against carbohydrates-containing moieties as they are often small and do not cause strong immune responses.

In a further aspect, carbohydrates can be attached to nanocrystals of cadmium selenide to provide quantum dots, which can then be guided to the required cellular structure by nanoparticles. Other anions such as sulphide may be used in addition to selenide. Quantum dots have potential uses in biological imaging, in both

electronic and optical devices, quantum computers and the screening of candidate drugs.

Embodiments of the present invention will now be described by way of example and not limitation with reference to the accompanying figures.

Brief Description of the Figures

Figure 1 shows the Zero-Field Cooling (ZFC, bold symbols) and the Field Cooling (FC, empty symbols) curves for *lacto*-AuFe glyconanoparticles (a) and the *malto*-AuFe glyconanoparticles (b).

Figure 2 shows transmission electron micrographs (left) and core size distribution histograms (right) for the *lacto*-AuFe glyconanoparticles (A) and the *malto*-AuFe glyconanoparticles (B).

Figure 3 depicts schematically the synthesis the magnetic glyconanoparticles.

Detailed Description

Pharmaceutical Compositions

The nanoparticles described herein or their derivatives can be formulated in pharmaceutical compositions, and administered to patients in a variety of forms. Thus, the nanoparticles may be used as a medicament for tumour targeting and hyperthermic therapies, for in vivo cell and tissue labelling, or as contrast enhancement media in magnetic resonance imaging.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an

adjuvant or an inert diluent. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. Such compositions and preparations generally contain at least 0.1wt% of the compound.

Parenteral administration includes administration by the following routes: intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraocular, transepithelial, intraperitoneal and topical (including dermal, ocular, rectal, nasal, inhalation and aerosol), and rectal systemic routes. For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, solutions of the compounds or a derivative thereof, e.g. in physiological saline, a dispersion prepared with glycerol, liquid polyethylene glycol or oils.

In addition to one or more of the compounds, optionally in combination with other active ingredient, the compositions can comprise one or more of a pharmaceutically acceptable excipient, carrier, buffer, stabiliser, isotonicizing agent, preservative or anti-oxidant or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may

depend on the route of administration, e.g. orally or parenterally.

Liquid pharmaceutical compositions are typically formulated to have a pH between about 3.0 and 9.0, more preferably between about 4.5 and 8.5 and still more preferably between about 5.0 and 8.0. The pH of a composition can be maintained by the use of a buffer such as acetate, citrate, phosphate, succinate, Tris or histidine, typically employed in the range from about 1 mM to 50 mM. The pH of compositions can otherwise be adjusted by using physiologically acceptable acids or bases.

Preservatives are generally included in pharmaceutical compositions to retard microbial growth, extending the shelf life of the compositions and allowing multiple use packaging. Examples of preservatives include phenol, meta-cresol, benzyl alcohol, para-hydroxybenzoic acid and its esters, methyl paraben, propyl paraben, benzalconium chloride and benzethonium chloride. Preservatives are typically employed in the range of about 0.1 to 1.0 % (w/v).

Preferably, the pharmaceutically compositions are given to an individual in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. Typically, this will be to cause a therapeutically useful activity providing benefit to the individual. The actual amount of the compounds administered, and rate and time-course of administration, will depend on the nature and severity of the condition being treated. Prescription of

treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 19th Edition, 1995. By way of example, and the compositions are preferably administered to patients in dosages of between about 0.01 and 100mg of active compound per kg of body weight, and more preferably between about 0.5 and 10mg/kg of body weight.

Antibodies

The nanoparticles may be used as carriers for raising antibody responses against the ligands linked to the core particles. These antibodies can be modified using techniques which are standard in the art. Antibodies similar to those exemplified for the first time here can also be produced using the teaching herein in conjunction with known methods. These methods of producing antibodies include immunising a mammal (e.g. mouse, rat, rabbit, horse, goat, sheep or monkey) with the nanoparticle(s). Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and screened, preferably using binding of antibody to antigen of interest. Isolation of antibodies and/or antibody-producing cells from an animal may be accompanied by a step of sacrificing the animal.

As an alternative or supplement to immunising a mammal with a nanoparticle, an antibody specific for the ligand

and/or nanoparticle may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, e.g. using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047. The library may be naive, that is constructed from sequences obtained from an organism which has not been immunised with any of the nanoparticles, or may be one constructed using sequences obtained from an organism which has been exposed to the antigen of interest.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a substantially homogenous population of antibodies, i.e. the individual antibodies comprising the population are identical apart from possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies can be produced by the method first described by Kohler and Milstein, Nature, 256:495, 1975 or may be made by recombinant methods, see Cabilly et al, US Patent No. 4,816,567, or Mage and Lamoyi in Monoclonal Antibody Production Techniques and Applications, pages 79-97, Marcel Dekker Inc, New York, 1987.

In the hybridoma method, a mouse or other appropriate host animal is immunised with the antigen by subcutaneous, intraperitoneal, or intramuscular routes to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the nanoparticles used for immunisation. Alternatively, lymphocytes may be immunised in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma

cell, see Goding, Monoclonal Antibodies: Principles and Practice, pp. 59-103 (Academic Press, 1986).

The hybridoma cells thus prepared can be seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high level expression of antibody by the selected antibody producing cells, and are sensitive to a medium such as HAT medium.

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the nanoparticles/ligands. Preferably, the binding specificity is determined by enzyme-linked immunoabsorbance assay (ELISA). The monoclonal antibodies of the invention are those that specifically bind to the nanoparticles/ligands.

In a preferred embodiment of the invention, the monoclonal antibody will have an affinity which is greater than micromolar or greater affinity (i.e. an affinity greater than 10^{-6} mol) as determined, for example, by Scatchard analysis, see Munson & Pollard, Anal. Biochem., 107:220, 1980.

After hybridoma cells are identified that produce neutralising antibodies of the desired specificity and affinity, the clones can be subcloned by limiting dilution procedures and grown by standard methods. Suitable culture media for this purpose include Dulbecco's Modified Eagle's Medium or RPM1-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumours in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

Nucleic acid encoding the monoclonal antibodies of the invention is readily isolated and sequenced using procedures well known in the art, e.g. by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies. The hybridoma cells of the invention are a preferred source of nucleic acid encoding the antibodies or fragments thereof. Once isolated, the nucleic acid is ligated into expression or cloning vectors, which are then transfected into host cells, which can be cultured so that the monoclonal antibodies are produced in the recombinant host cell culture.

Hybridomas capable of producing antibody with desired binding characteristics are within the scope of the present invention, as are host cells containing nucleic acid encoding antibodies (including antibody fragments) and capable of their expression. The invention also

provides methods of production of the antibodies including growing a cell capable of producing the antibody under conditions in which the antibody is produced, and preferably secreted.

Antibodies according to the present invention may be modified in a number of ways. Indeed the term "antibody" should be construed as covering any binding substance having a binding domain with the required specificity. Thus, the invention covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including synthetic molecules and molecules whose shape mimics that of an antibody enabling it to bind an antigen or epitope, here a carbohydrate ligand as defined herein.

Examples of antibody fragments, capable of binding an antigen or other binding partner, are the Fab fragment consisting of the VL, VH, Cl and CH1 domains; the Fd fragment consisting of the VH and CH1 domains; the Fv fragment consisting of the VL and VH domains of a single arm of an antibody; the dAb fragment which consists of a VH domain; isolated CDR regions and $F(ab')_2$ fragments, a bivalent fragment including two Fab fragments linked by a disulphide bridge at the hinge region. Single chain Fv fragments are also included.

A hybridoma producing a monoclonal antibody according to the present invention may be subject to genetic mutation or other changes. It will further be understood by those skilled in the art that a monoclonal antibody can be subjected to the techniques of recombinant DNA technology to produce other antibodies, humanised antibodies or chimeric molecules which retain the specificity of the

original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP 0 184 187 A, GB 2 188 638 A or EP 0 239 400 A. Cloning and expression of chimeric antibodies are described in EP 0 120 694 A and EP 0 125 023 A.

Experimental Section

A method of synthesising magnetic glyconanoparticles covalently bound to ligands was devised. By way of example, thiol derivatised neoglycoconjugates **1** and **2** of two significant oligosaccharides, the non-antigenic disaccharide maltose (Glcα(1→4)Glcβ1-OR) and the antigenic lactose (Galβ(1→4)Galβ1-OR), were prepared to functionalise in situ magnetic nanoparticles (Figure 3, scheme 1). The synthesis of the disulfides **1** and **2** was carried out by glycosidation of the conveniently protected maltose and lactose derivatives with 11-acetylthio-undecanol and 11-acetylthio-3,6,9-trioxa-undecanol, respectively.[12] Both linkers have been used to test the influence of their hydrophobic or hydrophilic nature in the properties of the whole material. Compounds **1** and **2** were isolated as disulfide forms, and used in this form for the preparation of gold-iron protected glyconanoparticles. The water-soluble glyconanoparticles **1-AuFe** (*malto*-AuFe) and **2-AuFe** (*lacto*-AuFe) were obtained in methanol/water mixtures using one-pot synthesis. $FeCl_3$ and $HAuCl_4$ in a ratio 1:4 were reduced with $NaBH_4$ in the presence of disulphides **1** or **2**. The protection of the metal core with the neoglycoconjugate monolayers results in highly stable and

bio-functional nanoclusters. They have been purified by means of centrifugal filtering and characterised by ^{1}H-NMR, UV-vis, ICP, TEM, EDX and SQUID.

Iron analysis of the particle, carried out by means of inductively coupled plasma-atomic emission spectrometry (ICP), indicated 0.27% and 2.81% iron content for **1-AuFe** and for **2-AuFe**, respectively. These data correspond to an average Au:Fe ratio of 5:0.1 and 5:1 respectively. Figure 1 shows Zero-Field Cooling and Field Cooling magnetisation curves obtained for the *lacto*-AuFe (A) and *malto*-AuFe (B) nanoparticles by means of Superconducting Quantum Interference Device (SQUID) between 5k and 300k in a field of 5000e. From the magnetic measurements it is inferred that both a superparamagnetic and ferromagnetic behaviour are present between 5k and 300k. SQUID measurements confirm the superparamagnetic character of the glyconanoparticles which have a blocking temperature (T_B) below 5K (Fig. 1) , which would be expected for a magnetic nanoparticle of 2nm diameter. The superparamagnetic component is clearly observed from

a) the partial fitting of the experimental thermal dependence of magnetisation to the Curie-Weiss law;

b) the partial dependence of the hysteresis loop (not shown) on the ration between the applied field and the temperature (H/T);

c) the difference between ZFC and FC curves.

Figure 2 shown transmission electron micrographs (left) and core-size distribution histograms (right) for the *lacto*-AuFe (A) and *malto*-AuFe (B) nanoparticles. Each black dot corresponds to a single particle. The dots are regularaly separated by the ligands (neoglycoconjugate) attached to the core and they form ordered monolayers.

The TEM was recoreded at a 200kV electron beam energy on a Philips CM200 microscope.

In the case of the **2-AuFe** sample (*lacto-AuFe*), the glyconanoparticles are dispersed, spherical and homogeneous. The mean diameter of the gold/iron cluster was evaluated to be 2 nm. A few isolated particles with a size of about 10 nm have been found in some regions of the grid, but these particles have not been included in the histogram. In the case of the sample **1-AuFe** (*malto*-AuFe), the glyconanoparticle presents a bimodal particle size distribution, as indicated by the corresponding histogram (Fig. 2B). Particles with a mean diameter of the gold/iron cluster about 2.5 nm and less than 1.5 nm have been found. Worthy of note is the spontaneous formation of aligned chains in extended regions of the grid, indicating an additional magnetostatic force (Fig. 2B). This behaviour could be attributed to dipole-dipole magnetic forces or quantum tunnelling among the nanoparticles. The aligned arrangement was not observed in the micrographs obtained for the **2-AuFe** nanodots, although a high ordered monolayer is observed.

Preparation

MaltoC_{11}SauFe: A solution of $FeCl_3$ (2 mg; 0.013 mmol; 0.25 equiv) in water (0.5 mL) was added to a solution of disulfide **1** (80 mg; 0.075 mmol; 3 equiv.) in MeOH (11.5 mL) followed by the addition of a solution of $HauCl_4$ (17 mg; 0.05 mmol; 1 equiv) in water (2 mL). $NaBH_4$ 1 M (52 mg; 1.38 mmol; 27.5 equiv) was then added in small portions with rapid stirring. The black suspension formed was stirred for an additional 2 h and the solvent removed under vacuum. The glyconanoparticles are insoluble in MeOH but soluble in water.

LactoEG$_4$SauFe: A solution of $FeCl_3$ (1 mg; 0.0065 mmol; 0.25 equiv) in water (0.25 mL) was added to a solution of disulfide **2** (70 mg; 0.07 mmol; 5.5 equiv.) in MeOH (12 mL) followed by the addition of a solution of $HAuCl_4$ (8 mg; 0.025 mmol; 1 equiv) in water (1 mL). $NaBH_4$ 1 M (26 mg; 0.69 mmol; 27.5 equiv) was then added in small portions with rapid stirring. The black suspension formed was stirred for an additional 2 h and the solvent removed under vacuum. The glyconanoparticles are insoluble in MeOH but soluble in water.

Purification: Purification was performed by centrifugal filtration. The crude product was dissolved in water (~15 mL) NANOpure and the solution was loaded into a centrifugal filter device (CENTRIPLUS YM30, MICROCON, MWCO= 30000), and subjected to centrifugation (3000 x g, 40 min). The dark glyconanoparticle residue was washed with MeOH and water and the process repeated several times until the starting material could no longer be detected by thin layer chromatography (TLC). The residue was dissolved in water and centrifuged several times to eliminate insoluble materials. The clear solution was lyophilised and the products obtained were free of salts and starting material (absence of signals from disulfide and Na^+ ions in ^1H and ^{23}Na NMR spectroscopy).

Characterization: TEM examination of the samples was carried out at 200KV (Philips CM200 microscope). A single drop (20μL) of the aqueous solutions of the Au/Fe glyconanoparticles were placed onto a copper grid coated with a carbon film. The grid was left to dry in air for several hours at room temperature. Particle size distributions of the Au/Fe clusters were evaluated from several micrographs using an automatic image analyser.

EDX analysis was performed with a Philips DX4 equipment attached to the microscope. ICP analysis was performed by Agriquem S.L. following PEC-009 protocol. UV spectra were obtained by a UV/vis Perkin Elmer Lambda 12 spectrophotometer. ^{1}H-NMR spectra were acquired on Bruker DRX-500 spectrometers and chemical shifts are given in ppm (δ) relative to D_2O.

1-AuFe: TEM: average diameter of metallic core, 1.5 and 2.5 nm.

ICP: 0.27 % Fe

UV (H_2O): λ= 500 nm, surface plasmon resonance

^{1}H-NMR (500 MHz, D_2O) δ: 5.32 (s, 1H, H-1'), 4.37 (s, 1H, H-1), 4.00-3.30 (m, 13H), 2.70 (s, 2H, CH_2S), 1.85-1.20 (m, 17H)

2-AuFe: TEM: average diameter of metallic core, 2 nm.

ICP: 2.81 % Fe

UV (H_2O): λ= 500 nm, surface plasmon resonance

^{1}H-NMR (500 MHz, D_2O) δ: 4.49 (brd, 1H, H-1'), 4.40 (brs, 1H, H-1), 4.10-3.30 (m, 23H), 2.92 (m, 0.5H)

In conclusion, the inventors have developed a simple methodology to prepare water-soluble, superparamagnetic nanoparticles covalently linked to antigenic oligosaccharides. The methodology can be extended to the preparation of hybrid nanoparticles incorporating carbohydrates and other molecules. Carbohydrate-receptor interactions can direct the magnetic glyconanoparticles to target cells and tissues allowing their selective labelling. These kind of polyvalent magnetic glyconanoparticles complements the scarcely available bioactive magnetic nanoparticles.[9][10][17] Their easy

preparation and purification, their small core size and their stability and solubility in physiologically conditions convert these tools in potential candidates for diagnostic, tumour targeting [15], hyperthermia [16], and magnetic resonance imaging [17] applications.

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Claims:

1. A magnetic nanoparticle having a core comprising passive metal atoms and magnetic metal atoms, wherein the ratio of passive metal atoms to magnetic metal atoms in the core is between about 5:0.1 and about 2:5, which core is covalently linked to a plurality of ligands.

2. The magnetic nanoparticle of claim 1, wherein the ratio of passive metal atoms to magnetic metal atoms in the core is between about 5:0.1 and about 5:1.

3. The magnetic nanoparticle of claim 1 or claim 2, wherein the passive metal is gold, platinum, silver or copper, and the magnetic metal is iron or cobalt.

4. The magnetic nanoparticle of claim 3, wherein the passive metal is gold and the magnetic metal is iron.

5. The magnetic nanoparticle of claim 4, wherein the ratio of gold atoms to iron atoms is about 5:0.1.

6. The magnetic nanoparticle of claim 4, wherein the ratio of gold atoms to iron atoms is about 5:1.

7. The magnetic nanoparticle of any one of the above claims, wherein the core of the nanoparticle has a diameter between 0.5 and 100nm.

8. The magnetic nanoparticle of any one of the above claims, wherein the core of the nanoparticle has a diameter of less than 2nm.

9. The nanoparticle of any one of the preceding claims, wherein the ligand comprises a carbohydrate group.

10. The nanoparticle of any one of the preceding claims, wherein the ligand comprises a polysaccharide, an oligosaccharide or a monosaccharide group.

11. The nanoparticle of any one of the preceding claims, wherein the ligand comprises a glycanoconjugate.

12. The nanoparticle of claim 11, wherein the glycanoconjugate is a glycolipid or a glycoprotein.

13. The nanoparticle of any one of the preceding claims, wherein the ligand is linked to the core via a sulphide group.

14. The nanoparticle of any one of the preceding claims, wherein the nanoparticle comprises a label.

15. The nanoparticle of claim 14, wherein the label is a fluorescent group or a radioactive isotope.

16. The nanoparticle of any one of the preceding claims, wherein the nanoparticle comprises a peptide.

17. The nanoparticle of any one of claims 1 to 15, wherein the nanoparticle comprises DNA.

18. The nanoparticle of any one of claims 1 to 15, wherein the nanoparticle comprises RNA.

19. The nanoparticle of any one of the preceding claims, wherein the nanoparticle comprises a pharmaceutically active component.

20. The nanoparticle of claim 19, wherein the ligand binds a corresponding receptor on a cell.

21. The nanoparticle of any one of the preceding claims, wherein the nanoparticle is water soluble.

22. A composition comprising a population of one or more of the nanoparticles of any one of claims 1 to 21.

23. The composition of claim 22 which comprises a plurality of nanoparticles having different ligand groups.

24. A composition comprising a population of one or more of the nanoparticles of any one of claims 1 to 21 for use in a method of medical treatment.

25. The composition of any one of claims 22 to 24, which composition is a colloid.

26. The colloid of claim 25, wherein the nanoparticles have a mean diameter of less than 2nm.

27. The colloid of claim 25 or claim 26, which colloid is stable for at least about 1 year.

28. Use of a nanoparticle of any one of claims 1 to 21 or a composition of any one of claims 22 to 27 for the preparation of a medicament for the treatment of a condition ameliorated by the administration of the ligand.

29. The use of claim 28, wherein the ligand inhibits a carbohydrate mediated interaction that would otherwise

cause a pathology.

30. The use of claim 28 or claim 29, wherein the nanoparticle has a plurality of ligands attached thereto so that it is capable of inhibiting polyvalent carbohydrate mediated interactions.

31. Use of a nanoparticle of any one of claims 1 to 21 or a composition of any one of claims 22 to 27 for the preparation of a medicament for vaccinating a patient with an antigen, wherein the ligand linked to the core of the nanoparticle comprises the antigen.

32. Use of a nanoparticle of any one of claims 1 to 21 or a composition of any one of claims 22 to 27 for the preparation of a medicament for vaccinating a patient with nucleic acid encoding an antigen, wherein the ligand linked to the core of the nanoparticle comprises the nucleic acid.

33 Use according to claim 31 or 32 wherein the vaccine is administered by application of a magnetic field.

34. Use of a nanoparticle of any one of claims 1 to 21 or a composition of any one of claims 22 to 27 as a contrast agent in magnetic resonance imaging.

35. Use of a nanoparticle of any one of claims 1 to 21 or a composition of any one of claims 22 to 27 for the preparation of a medicament for the treatment of a tumour.

36. Use according to claim 35, wherein the tumour is exposed to a high frequency magnetic field.

37. Use according to claim 35, wherein the tumour is exposed to infrared light.

38. A method of preparing a nanoparticle having a core comprising gold atoms and iron atoms, which core is covalently linked to a plurality of ligands, the method comprising:

synthesizing a sulphide derivative of the ligand;

reacting the sulphide derivatised ligand with a ferric salt and $HAuCl_4$ in the presence of reducing agent to produce the nanoparticles.

39. The method of claim 38, wherein the ligand is derivatised as a protected disulphide.

40. The method of claim 38 or claim 39, wherein the ligand comprises a carbohydrate group.

41. A nanoparticle as obtainable by the method of any of claims 38 to claim 40.

42. A method of disrupting an interaction between a carbohydrate and a binding partner, the method comprising contacting the carbohydrate and the binding partner with nanoparticles according to any one of claims 1 to 21, wherein the ligands bound to the nanoparticles comprise a carbohydrate group capable of disrupting the interaction of the carbohydrate and the binding partner.

43. A method of screening for substances capable of binding to a ligand, the method comprising (a) contacting the nanoparticles of any one of claims 1 to 21 with one or more candidate compounds and (b) determining whether

the candidate compounds binds to the ligand.

44 The method of claim 43, wherein the ligand comprises a carbohydrate group.

45. A method of determining the presence in a sample of a substance capable of binding to a ligand, the method comprising (a) contacting the sample with the nanoparticles of any one of claims 1 to 21 so that the substance binds to the ligand of the nanoparticles and (b) determining whether binding takes place.

46 The method of claim 45, wherein the ligand comprises a carbohydrate group.

47. The method of claim 45 or claim 46, further comprising the step of correlating the presence or absence of binding with the diagnosis of a disease state associated with the presence of the substance.

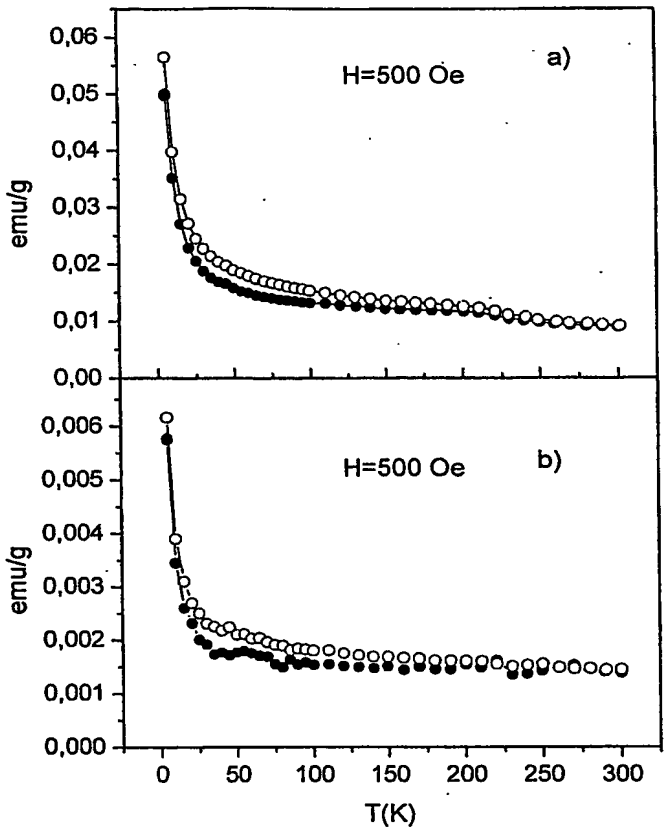
48. The method of any one of claims 45 to 47, wherein the substance is an antibody which is capable of binding to the ligand.

49. A method of determining whether a carbohydrate mediated interaction occurs, the method comprising (a) contacting one or more species suspected to interact via a carbohydrate mediated interaction with the nanoparticles of any one of claims 1 to 21 and (b) determining whether the nanoparticles modulate the carbohydrate mediated interaction.

50. The method of any one of claims 43 to 49, wherein the nanoparticles are detected by nuclear magnetic

resonance (NMR), aggregation, transmission electron microscopy (TEM), atomic force microscopy (AFM), surface plasmon resonance (SPR), or with nanoparticles comprising silver atoms, signal amplification using the nanoparticle-promoted reduction of silver (I).

FIG 1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b)
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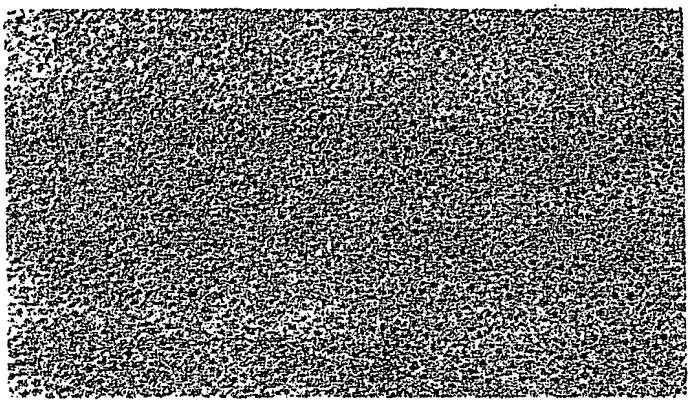
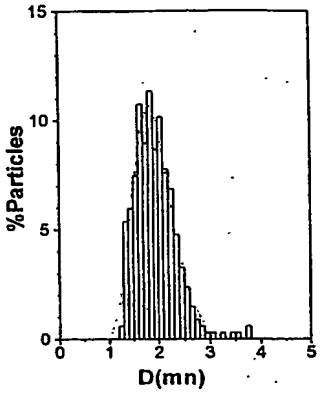
300
T(K)

FIG 2

A)

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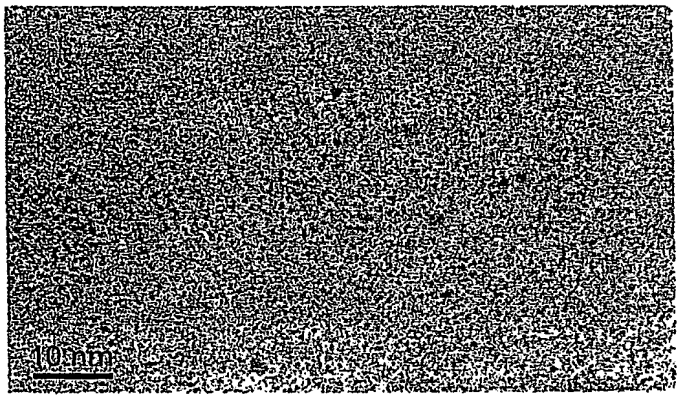
D(mn)

B)


10 nm

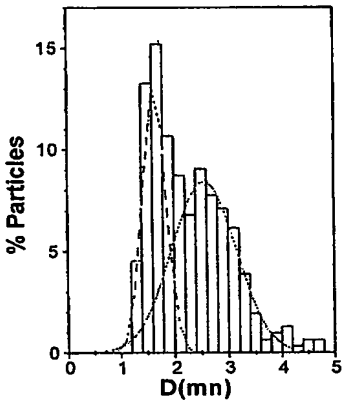

15
10
5
0
% Particles
0
1
2
3
4
5
D(mn)

FIG 3